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Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation

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Objective. **Pluripotent hematopoietic stem cells and muscle-derived hematopoietic potential cells isolated by Hoechst 33342 dye-mediated fluorescein-activated cell sorting (FACS) as side population (SP) cells, give rise to hematopoietic cells as well as skeletal muscle cells following intravenous transplantation. However, besides bone marrow and skeletal muscle, it has remained unclear whether other adult tissues also contain SP cells that are enriched for cells that exhibit hematopoietic potential.**

Methods. **To test whether adult tissues contain SP cells with hematopoietic potential, Hoechst-FACS analysis and hematopoietic colony formation assays were performed with cells isolated from a variety of adult tissues, skeletal muscle, heart, brain, spleen, liver, kidney, lung, and small intestine and compared with peripheral blood and bone marrow cells.**

Results. **In addition to hematopoietic tissues, cell preparations from nonhematopoietic tissues, such as skeletal muscle, kidney, lung, and small intestine, displayed markedly higher hematopoietic colony formation activity compared to peripheral blood cells. Moreover, the hematopoietic progenitors in these adult tissues expressed the hematopoietic cell marker CD45. Hoechst-FACS analysis demonstrated that all adult tissues examined contained SP cells. In addition, these SP fractions were enriched for cells that efficiently formed hematopoietic colonies in vitro.**

Conclusion. **These results indicate that hematopoietic progenitors are present in significant numbers in all adult tissues examined.**

During embryogenesis, the formation of primitive blood cells begins in the yolk sac on embryonic day 7.5 (E7.5). Thereafter, definitive hematopoietic stem cell (HSC) activity is first detectable in the aorta-gonad-mesonephros (AGM) region on E10, followed by fetal liver and yolk sac. The fetal liver by E12 is the main site of definitive hematopoiesis. HSC in the fetal liver subsequently migrates to the bone marrow, which becomes the major site of hematopoiesis throughout normal adult life [1]. However, recent experiments have revealed that adult liver also contains hematopoietic stem cells (HSCs) that can reconstitute hematopoiesis in lethally irradiated animals [2]. One possibility is that a small number of HSCs in fetal liver remains in the liver in adulthood. Alternatively, the HSCs in adult liver may be derived from bone marrow. At present, it is still unclear which is the case.

Recent experiments have identified the existence of adult stem cells present in several tissues that appear to exhibit the ability to differentiate into many different cell types following reintroduction in vivo. This work has raised important questions regarding the developmental potential of stem cells derived from diverse tissues including muscle, bone marrow, and brain [3,4]. Neural stem cells (NSCs) derived from the adult central nervous system were suggested to give rise to hematopoietic cells following intravenous injection [5,6] and to different embryonic tissues after introduction into embryo [7]. However, primary NSCs failed to exhibit any hematopoietic potential whatsoever [8]. Bone marrow–derived cells exhibit the potential to give rise to a wide variety of cell types, such as skeletal muscle [9] and neuronal cells [10–12]. Moreover, HSCs purified from bone marrow exhibit plasticity when introduced into different tis-

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sues. For example, HSCs can differentiate into skeletal myocytes [13], hepatic cells [14], cardiac muscle, and vascular endothelium [15] following transplantation. Animals transplanted with a single HSC contained extensive contribution of donor-derived cells to epithelial cell types [16]. Similarly, many or all tissues appear to contain a population of adult stem cells that differentiate in a context-specific manner, presumably in response to growth factors and signals provided by their host tissues [3]. However, demonstration of pluripotency by clonal cell transplantation experiments has yet to be performed.

Recent experiments demonstrated that HSCs in bone marrow from many different species can be purified by fluorescein-activated cell sorting (FACS) of side population (SP) cells [17–19]. The SP fraction of cells excludes Hoechst dye through the activity of multidrug resistance (MDR)-like proteins such as BCRP1 on the cell surface [20,21]. Muscle-derived hematopoietic stem cells exhibit the capacity to reconstitute the entire hematopoietic repertoire following intravenous injection into lethally irradiated mice [22,23]. Recent work suggested that cells with hematopoietic potential within skeletal muscle are a bone marrow origin [23,24]. Muscle-derived hematopoietic potential cells can be isolated by the Hoechst-FACS method as muscle SP cells. In addition to hematopoietic potential, transplanted SP cells isolated from bone marrow or muscle actively participate in the formation of skeletal myotubes during regeneration [13]. However, the definitive plasticity of these cells that give rise to hematopoietic and muscle cells has to be confirmed from single cell experiments. Nevertheless, it is interesting to note that tissue-derived adult stem cells have common properties such as high level of Hoechst dye exclusion and hematopoietic potential. However, it has remained unclear whether SP cells are also present in other adult tissues and whether any other adult tissues contain cells with hematopoietic potential.

Here we demonstrate that diverse nonhematopoietic tissues contain SP cells and that the SP fraction was enriched for cells that efficiently formed hematopoietic colonies in vitro. Moreover, the SP cells that exhibited this hematopoietic potential expressed the definitive hematopoietic lineage marker CD45. These results suggest that hematopoietic progenitors are widespread in many adult nonhematopoietic tissues and can be enriched by isolation of the Hoechst 33342–effluxing fraction of SP cells.

Materials and methods

Cell preparations

Cell suspensions of tissues were prepared from hind limb skeletal muscle, whole brain, spleen, heart, liver, lung, small intestine, and kidney of adult Balb/c mice (1- to 2-month-old), as previously described [25]. Briefly, minced tissues were digested with 10 mg/mL collagenase type B (Roche Diagnostics, Laval, PQ, Canada), 2.4 U/mL dispase II (Roche Diagnostics), and 2.5 mM $CaCl_2$ at 37°C for 20 minutes (skeletal muscle) or 10 minutes (other tissues), followed by filtration with Netwell filter (74 μm pore size, Fisher Scientific, Nepean, ON, Canada). Bone marrow was prepared as described previously [17]. Peripheral blood was collected from tails of mice.

Hematopoietic progenitor colony assay

One to 5 × 10^4 nucleated cells prepared from whole tissues or FACS-sorted cells were cultured in Methocult M3434 (Stem Cell Technologies, Vancouver, BC, Canada). Hematopoietic colonies consisting of more than 50 cells were scored at 14 days after the cultures were initiated. Hematopoietic colony types were confirmed by cytocentrifuge, and staining with May-Grunwald Giemsa (Sigma-Aldrich, Oakville, ON, Canada) or by immunohistochemistry with Mac-1 and Gr-1 antibodies (BD Pharmingen, Mississauga, ON, Canada) for detection of granulocyte/monocyte followed by Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA).

Fluorescein-activated cell sorting (FACS)

Hoechst staining and FACS analysis was performed as described previously [17,22]. FACS analysis was performed on MoFlo (Cytomation Inc., Fort Collins, CO, USA) equipped with dual lasers. Hoechst staining was performed in DMEM supplemented with 2% fetal calf serum (FCS), 10 mM HEPES, 5 μg/mL (for bone marrow, skeletal muscle, brain, and heart) or 10 μg/mL (for liver, spleen, kidney, lung, and small intestine) Hoechst 33342 (Sigma-Aldrich) and with or without 50 μM verapamil (Sigma-Aldrich) at 37°C for 90 minutes. Following Hoechst staining, immunostaining was performed by using antibodies reactive to CD45 conjugated with fluorescein isothiocyanate (FITC; BD Pharmingen). Hoechst dye was excited at 351 nm by UV laser and its fluorescence detected at two wavelengths using 424/44 (Hoechst Blue) and 675/LP (Hoechst Red) filters. FITC was excited at 488 nm by argon laser and its fluorescence detected at FL1 (530/40) filter. Dead cells were excluded from the plots based on PI (propidium iodide, Sigma-Aldrich) staining (2 μg/mL). Viability of sorted cells was assessed following 0- and 24-hours cultures. Briefly, 1–5 × 10^4 sorted live cells unstained with trypan blue (Invitrogen) were plated in Methocult M3434 and then cells were harvested from the cultures following 0 (control) and 24 hours culture. Trypsin (Invitrogen) was also used for recovery of adherent cells. Live cells were counted for the calculation of cell viability: cell viability (%) = cell number of 24 hours culture/cell number of 0 hour culture × 100. The cell viabilities following 24 hours culture were within a range from 30 to 60%.

Results

To test whether cells prepared from nonhematopoietic adult tissues exhibit hematopoietic potential in vitro, hematopoietic progenitor colony formation assays were performed using whole cells enzymatically digested from adult skeletal muscle, brain, liver, spleen, kidney, heart, lung, and small intestine, as well as peripheral blood and bone marrow cells. In vitro hematopoietic colony formation activity of dissociated cells was determined by plating the cells in methylcellulose medium, Methocult M3434, containing stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6), and erythropoietin (EPO), permitting the clonal growth of myeloid type of hematopoietic cells [26]. Culture of dissociated cells derived from all adult tissues examined gave rise to a

variable frequency of hematopoietic colonies (Fig. 1). May-Grunwald Giemsa staining and immunohistochemical staining against myeloid cell markers, Mac-1 and Gr-1, confirmed that these colonies contained hematopoietic cells (data not shown). In contrast, no hematopoietic colonies were detected following plating of 5×10^4 nucleated peripheral blood cells, ruling out the possibility that tissue-derived hematopoietic colonies were from contaminating peripheral blood cells in the tissue preparation (Fig. 1). The highest incidence of hematopoietic colonies was in bone marrow (238.3 ± 36.9 per 5×10^4 input nucleated cells). Cells prepared from skeletal muscle formed relatively higher incidence of hematopoietic colonies (81 ± 7.5 per 5×10^4 nucleated cells). Cells prepared from liver, spleen, kidney, lung, and small intestine formed moderate numbers of hematopoietic colonies (33 ± 3.6, 55 ± 4.6, 29.3 ± 2.1, 58.3 ± 4.9, and 45.3 ± 9.6 per 5×10^4 nucleated cells, respectively). Cells prepared from adult brain and heart contained lower but significant levels of hematopoietic colonies (4.3 ± 0.6 and 7.3 ± 2.1 per 5×10^4 nucleated cells, respectively). Heart is a highly vascularized tissue that exhibited relatively low numbers of hematopoietic colonies, supporting the notion that tissue-derived hematopoietic colonies were not derived from contaminating peripheral blood cells in the tissue preparation.

To investigate the origin of the hematopoietic progenitors in these adult tissues, FACS analysis was employed by sorting $CD45^+$ cells from the nucleated cells prepared from adult tissues (Table 1). As anticipated, bone marrow and spleen contained high levels of CD45-expressing cells (71.0% and 89.5%, respectively). Liver, lung, and skeletal muscle also contained moderate levels of CD45-expressing cells (27.0%, 38.0%, and 22.4%, respectively). Brain, kidney, heart, and small intestine contained relatively lower levels of CD45-expressing cells (5.5%, 9.6%, 3.0%, and 9.1%, respectively). These $CD45^+$ cells were present in the low to middle forward scatter (FSC) and the low side scatter (SSC) fraction (data not shown). Notably, the hematopoietic progenitor activity in these adult tissues was exclusively fractionated into the $CD45^+$ fraction of cells.

The hematopoietic colonies derived from adult tissues contained various types of colonies (Table 1). Most colonies consisted of granulocytes and/or monocytes only. However, similar to bone marrow, cells prepared from skeletal muscle and spleen also formed mixed colonies containing erythroid cells, myeloid cells, and megakaryocytes, indicating that both tissues contained primitive myeloid progenitors (GEMM), while the other tissues simply contained more committed myeloid progenitors. Therefore, these results strongly suggest that $CD45^+$ hematopoietic progenitors are present within nonhematopoietic tissues such as skeletal muscle. In addition, among nonhematopoietic adult tissues, only skeletal muscle contained GEMM, suggesting unique muscle environment that allows most primitive progenitors to survive.

Pluripotent HSCs and muscle-derived hematopoietic-potential cells, which are purified by Hoechst dye–mediated FACS analysis as SP cells, can give rise to hematopoietic cell lineages as well as skeletal muscle cells, following intravenous transplantation [13]. We utilized Hoechst/FACS method to test whether other adult tissues also contained SP cells. We first examined Hoechst staining with different concentration of the dye to find the optimal staining condition for obtaining clear SP compartments in cells derived from various adult tissues (data not shown). As a result, optimal results were obtained when 5 μg/mL Hoechst 33342 was used for staining for bone marrow, skeletal muscle, brain, and heart, and 10 μg/mL Hoechst 33342 was used for liver, spleen, kidney, lung, and small intestine. Interestingly, not only bone marrow and skeletal muscle but also other tissues examined contained different proportions of SP cells (Fig. 2). Compared to marrow-derived SP cells (0.79% of total nucleated cells), similar numbers of SP cells were detected in spleen and lung (0.96% and 0.98% of total nucleated cells, respectively). Skeletal muscle, liver, kidney, heart, and small intestine contained much higher numbers of SP cells (3.1%, 4.3%, 5.8%, 9.1%, and 8.6% of total nucleated cells, respectively). The highest number of SP cells was brain (15.1% of total nucleated cells recovered). All of the SP cells, except for a subpopulation of brain SP cells, stained with Hoechst dye were commonly sensitive to verapamil, suggesting that the efflux of Hoechst dye in these SP cells is mediated by the activity of MDR-like proteins such as BCRP1 on the cell surface [20,21]. Brain SP cells contained relatively higher verapamil-resistant cells (1/3 of SP cells), suggesting that verapamil-resistant MDR-like protein or a different mechanism may be involved in the dye efflux activity of a subpopulation of brain SP cells. In contrast, no SP cells were detected in nucleated peripheral blood cells, ruling out the possibility that tissue-derived SP cells were from contaminating peripheral blood cells in the tissue preparation (data not shown). Therefore, these re-

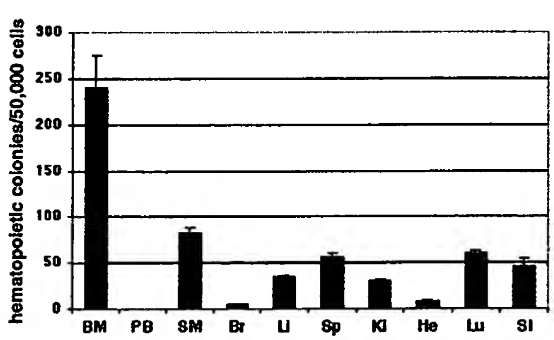

Figure 1. Cells prepared from adult tissues contain hematopoietic progenitors. 5×10^4 nucleated cells prepared from bone marrow (BM), peripheral blood (PB), skeletal muscle (SM), brain (Br), liver (Li), spleen (Sp), kidney (Ki), heart (He), lung (Lu), and small intestine (SI) were cultured in Methocult M3434 for14 days. Cells prepared from all adult tissues examined but not from peripheral blood formed hematopoietic colonies. The error bars represent the standard error of the mean from triplicated samples. Similar results were obtained from at least three independent experiments.

Table 1. Hematopoietic colony formation from different adult tissues

	Bone Marrow (1×10^4)				Skeletal Muscle (2×10^4)				Brain (2×10^4)			
		Colony				Colony				Colony		
Fraction	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E
Whole	100	5.5	38.5	8.0	100	1.5	17.5	1.0	100	0	2.0	0
$CD45^-$	29.0	0	3.0	1.0	77.6	0	1.0	0	94.5	0	0	0
$CD45^+$	71.0	4.0	33.5	6.0	22.4	5.0	27.0	2.5	5.5	0	23.5	0
	Liver (2×10^4)				Spleen (2×10^4)				Kidney (2×10^4)			
		Colony				Colony				Colony		
Fraction	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E
Whole	100	0	9.0	0	100	3.0	7.0	1.0	100	0	100.2	0
$CD45^-$	73.0	0	1.5	0	10.5	0	3.0	0.5	90.4	0	1.5	0
$CD45^+$	27.0	0	8.0	0	89.5	3.5	11.5	0	9.6	0	65.0	0
	Heart (2×10^4)				Lung (2×10^4)				Small Intestine (2×10^4)			
		Colony				Colony				Colony		
Fraction	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E	%	GEMM	GM	BFU-E
Whole	100	0	1.5	0	100	0	21.0	0	100	0	16.5	0
$CD45^-$	97.0	0	0	0	61.8	0	0	0	90.0	0	2.0	0
$CD45^+$	3.0	0	8.0	0	38.2	0	27.5	0	9.1	0.5	60.5	0

1×10^4 (bone marrow–derived) or 2×10^4 (other tissues) unfractioned whole cells, $CD45^-$ and $CD45^+$ cells, prepared from adult tissues, were cultured in methylcellulose (Methocult M3434) for 14 days. Second column indicates percentage (%) of $CD45^-$ and $CD45^+$ cells in adult tissues. GEMM, mixed colonies containing erythroid cells, myeloid cells, and megakaryocytes; GM, granulocytes and/or monocytes colonies; BFU-E, erythroid burst-forming units. Data represent the mean from duplicated samples. Similar results were obtained from at least three independent experiments.

sults indicate that, similar to bone marrow and skeletal muscle, all adult tissues examined contain SP cells that presumably exhibit a high level of MDR-like activity.

We have demonstrated that all tissues examined contain different degrees of hematopoietic progenitors that readily form hematopoietic colonies in vitro. To test whether these hematopoietic progenitors were fractionated into the SP compartment, the nucleated cells prepared from adult tissues were sorted by FACS into SP and MP (main population) fractions. Then, we cultured the sorted fractions in Methocult M3434 to score hematopoietic colony formation. Interestingly, SP cells derived from all adult tissues examined efficiently formed numerous hematopoietic colonies in vitro (Fig. 3). By contrast, MP cells derived from all examined adult tissues gave rise to markedly fewer hematopoietic colonies per 1×10^4 input nucleated cells, compared to SP cells. The different hematopoietic progenitor activities per input cells between SP and MP cells derived from adult tissues are not due to the different cell survival rate between two type of cells during culture following cell sorting by FACS, as cell survival rate in Methocult M3434 was not significantly different between SP and MP cells by 24 hours after sorting (data not shown). The highest hematopoietic colony formation activity was found in cultures of bone marrow SP cells (155 ± 19.9 per 1×10^4 nucleated cells). Skeletal muscle SP cells displayed relatively higher incidence of hematopoietic colonies (44.7 ± 1.5 per 1×10^4 nucleated cells). Since skeletal muscle SP cells also contain enriched muscle-derived hematopoietic potential cells that can differentiate into hematopoietic cells following transplantation [13], these results suggest that the hematopoietic progenitors in bone marrow and skeletal muscle possess similar biological features to HSCs and muscle-derived hematopoietic potential cells that have high level of MDR activity [20,27]. Hematopoietic progenitors in liver were also efficiently fractionated into SP cells (30.3 ± 0.6 per 1×10^4 nucleated cells), suggesting that HSCs present in adult liver may be purified as SP cells. Spleen, kidney, lung, and small intestine SP cells also displayed relatively higher incidence of hematopoietic colonies (45 ± 0.6, 20.3 ± 1.2, 33.3 ± 1.2, and 30.3 ± 1.5 per 1×10^4 nucleated cells, respectively). Brain and heart SP cells contained low but significantly higher numbers of hematopoietic progenitors (11.7 ± 0.6 and 3.3 ± 0.6 per 1×10^4 nucleated cells, respectively), compared to total unfractionated nucleated cells (0.9 ± 0.1 and 1.4 ± 0.4 per 1×10^4 nucleated cells, respectively) or MP cells (0.7 ± 1.2 and 0.3 ± 0.6 per 1×10^4 nucleated cells, respectively). The MP cells derived from these adult

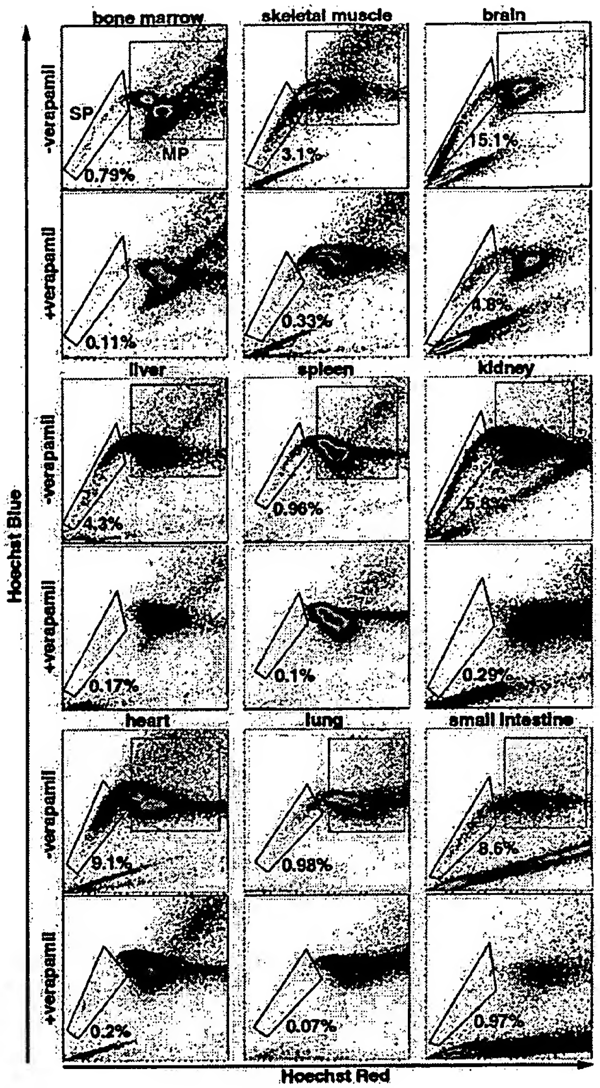

Figure 2. Adult tissues contain SP cells. Hoechst dye–mediated FACS analysis was employed for the detection of SP cells in nucleated cells prepared from adult tissues. The SP cells are indicated in enclosed black-lined boxes and the percentage of cells in this region is indicated in each panel. MP cells are indicated in enclosed red-lined boxes. Most of the SP cells stained with Hoechst dye were sensitive to verapamil. The data are representative of at least three experiments.

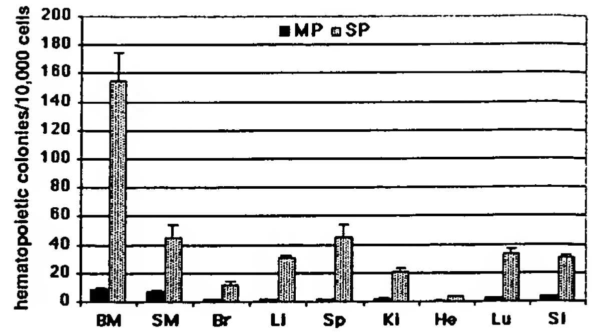

Figure 3. SP cells derived from adult tissues contain enriched hematopoietic progenitors. 1 × 10^4 nucleated sorted MP and SP cells prepared from bone marrow (BM), skeletal muscle (SM), brain (Br), liver (Li), spleen (Sp), kidney (Ki), heart (He), lung (Lu), and small intestine (SI) were cultured in Methocult M3434 for14 days. All adult tissues examined efficiently gave rise to hematopoietic colonies of SP cells (gray bars) relative to MP cells (black bars). The error bars represent the standard error of the mean from triplicated samples. Similar results were obtained from at least three independent experiments.

tissues also gave rise to hematopoietic colonies (Fig. 3 and Table 2), indicating that hematopoietic progenitors were fractionated not only into SP but also into MP compartments. However, these colonies derived from all MP cells were more committed colonies containing only granulocytes or monocytes. By contrast, in the case of bone marrow and skeletal muscle, GEMM colonies derived from primitive myeloid progenitors were observed to form only from the SP fraction (data not shown).

SP cells derived from adult tissues contained various proportions of CD45-expressing cells (Table 3). Hematopoietic progenitor activity of the SP cells derived from these adult tissues correlated with the proportion of CD45-expressing cells within the SP compartment. For example, heart and brain contained relatively lower proportions of $CD45^+$ cells within their SP compartments. The SP cells derived from both tissues gave rise to a lower frequency of hematopoietic colonies compared to other tissues. In addition, the hematopoietic progenitor activities of SP cells derived from bone marrow, skeletal muscle, and brain exclusively fell into $CD45^+$ fractions (data not shown). Taken together, these results strongly suggest that hematopoietic progenitors are present in many adult nonhematopoietic tissues and can be isolated as Hoechst 3334–effluxing SP cells.

Discussion

Recent experiments demonstrate that HSCs from bone marrow and hematopoietic potential cells from skeletal muscle can be purified by FACS of SP cells on the basis of Hoechst dye exclusion [13,17]. Stem cells from marrow and muscle exhibit the capacity to reconstitute the entire hematopoietic repertoire following intravenous injection into lethally irradiated mice [22–24]. Our experiments revealed the presence of SP cells in all adult tissues examined. In addition, this work documents that tissue-derived SP cells contain a $CD45^+$ subpopulation that exhibits hematopoietic progenitor activity in vitro.

Hematopoiesis has previously been demonstrated to occur in several tissues besides bone marrow. For example, hematopoietic stem cells and progenitors readily migrate to and colonize the adult spleen [28]. In addition, adult lung contains abundant numbers of alveolar macrophages derived from progenitors identified in fetal lung [29]. Recently, adult liver has been shown to contain hematopoietic

Table 2. Proportion of total hematopoietic progenitors in SP vs MP fractions

Fraction	Bone Marrow	Skeletal Muscle	Brain	Liver	Spleen	Kidney	Heart	Lung	Small Intestine
MP (%)	85.6	82.3	24.3	49.5	60.4	51.6	50.0	83.5	45.0
SP (%)	14.4	17.7	75.7	50.5	39.6	48.4	50.0	16.5	55.0

Each number indicates percentage of proportion of total hemtopoietic progenitors in SP vs MP fractions derived from adult tissues. Data represent the mean from triplicated samples. Similar results were obtained from at least three independent experiments.

stem cells that reconstitute hematopoiesis in lethally irradiated animals [2]. Furthermore, T cells can differentiate in extrathymic sites, such as intestine and liver [30,31]. Our experiments have revealed that these and other tissues contain SP cells that are also a rich source of hematopoietic progenitors. Taken together, these data suggest the hypothesis that significant numbers of hematopoietic progenitors are normally located outside the marrow.

Muscle satellite cells are the committed stem cells of adult skeletal muscle [4,25,32]. Similarly, NSCs are stem cells for the adult central nervous system [33]. Interestingly, skeletal muscle SP cells do not express any satellite cell markers such as Myf5 or desmin (data not shown). Similarly, brain SP cells do not express a NSC marker, nestin (data not shown). Importantly, muscle SP cells are present in *Pax7*-deficient muscle that entirely lacks myogenic satellite cells but contains high levels of hematopoietic progenitor activity [34]. Taken together, these data argue that adult stem cells purified into SP cells represent a class of stem cells distinct from tissue-specific stem cells such as muscle satellite cells and NSCs.

We observed that cells with in vitro hematopoietic progenitor potential, isolated from adult tissues, efficiently segregate to the SP fraction and express the hematopoietic marker CD45. Recent experiments demonstrated that skeletal muscle–derived hematopoietic stem cells are indeed $CD45^+$ [23,24]. Taken together, these results can be interpreted to support the assertion that HSCs derived from the bone marrow populate diverse tissues during normal development. Alternatively, it can be hypothesized that adult stem cells represent a more primitive class of stem cells that also expresses CD45 but is upstream of HSCs.

Our experimental data indicate that the SP fraction of cells isolated from diverse adult tissues contains short-term hematopoietic progenitors that can be readily assayed using an in vitro differentiation assay. However, only bone marrow, skeletal muscle, and spleen contained primitive myeloid progenitors (GEMM), while the other tissues simply contained more committed myeloid progenitors. In addition, in the case of bone marrow and skeletal muscle, the GEMM were observed to form only from the SP fraction. These results may imply something unique about the skeletal muscle environment that allows it to support survival of the most primitive progenitors. In addition to bone marrow and muscle SP cells, the SP fraction from human fetal liver has also been demonstrated to contain in vivo hematopoietic activity [19]. These results suggest that short-term hematopoietic progenitors including primitive myeloid progenitors and long-term hematopoietic stem cells similarly possess high levels of MDR-like activity [27]. SP cells are also present in human umbilical cord blood (UCB) [18], which is used as an alternative source of HSCs for transplantation purposes [35]. UCB-derived SP cells contain both short-term and long-term hematopoietic progenitors [36]. However, the definitive presence of stem cells in adult tissues other than marrow, liver, brain, and muscle with long-term hematopoietic reconstituting ability remains to be ascertained. Recently, SP cells have been shown to exist in several cultured cells such as embryonic stem cells and neurosphere cells that containing neural stem cells [20,37]. It is unlikely that these SP cells have hematopoietic potential. However, they appear to possess more potent characteristics as a progenitor than the non-SP cells.

Adult stem cells have been suggested to represent a population of pluripotential stem cells that differentiate in a context-specific manner, presumably in response to local growth factors and signals provided by their host tissues [3,16]. Indeed, muscle SP cells differentiate into skeletal muscle both in vitro and in vivo ([13] and data not shown). In addition, adult stem cells isolated from the liver have been demonstrated to differentiate into biliary hepatic epithelial cells in vitro [38]. Therefore, the presence of putative HSC-like stem

Table 3. Percentages of $CD45^+$ cells in SP cells derived from different adult tissues

Fraction	Bone Marrow	Skeletal Muscle	Brain	Liver	Spleen	Kidney	Heart	Lung	Small Intestine
$CD45^-$ (%)	15.6	75.1	93.9	61.0	6.3	58.8	93.2	79.3	77.0
$CD45^+$ (%)	84.4	24.9	6.1	39.0	93.7	41.2	6.8	20.7	23.0

FACS analysis was employed for the detections of $CD45^+$ cells in SP cells prepared from adult tissues. Each number indicates percentage of $CD45^-$ and $CD45^+$ cells in SP gates shown in Figure 3. Data represent one experiment. Similar results were obtained from at least three independent experiments.

cells in adult tissues raises the possibility that such stem cells locally contribute to host tissues when exposed to the correct environment during regeneration. Clearly, additional experimentation is required to investigate the origin and biological significance of the SP cells with hematopoietic potential within nonhematopoietic tissues.

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